

## CLAIMS

### WHAT IS CLAIMED IS:

1. A method of detecting at least first and second target molecules in a sample comprising:
  - a. contacting said first and second target molecule with a composition comprising:
    - i. an amplification enzyme; and
    - ii. first and second target probes, said first and second target probes comprising:
      - a) a first and second bioactive agent, respectively, wherein said first and second bioactive agents specifically bind to said first and second target molecules, respectively;
      - b) a first and second adapter sequence, respectively, wherein said first adapter sequence identifies said first target molecule and said second adapter sequence identifies said second target molecule; and
      - c) at least first and second upstream universal priming sequences;
  - b. amplifying said first and second adapter sequences using said first and second universal priming sequences, wherein no ligation is performed, to form first and second amplicons, respectively;
  - c. detecting said first and second amplicons, respectively, to indicate the presence or absence of said first and second target molecules in said sample.
2. The method according to claim 1, wherein said first and second target molecules are selected from the group consisting of proteins and nucleic acids.
3. The method according to claim 2, wherein said first and second target molecules are nucleic acids.
4. The method according to claim 3, wherein said first and second bioactive agents are first and second target specific domains that are substantially complementary to at least one domain of said first and second target nucleic acids, respectively.
5. The method according to claim 4, wherein said first and second target nucleic acids further comprise a first and second detection position, respectively, and said first and second target specific domains comprise a nucleotide at a readout position that is perfectly complementary to the nucleotide at said first and second detection positions.
6. The method according to claim 4, wherein said first and second upstream universal priming sequences are RNA Polymerase primers and said enzyme is an RNA Polymerase.
7. The method according to claim 6, wherein said RNA Polymerase primers are T7 primers.

8. The method according to claim 4, further comprising contacting said first and second upstream universal priming sequences with first and second chimeric RNA/DNA primers, wherein said amplification is by SPIA.

9. The method according to claim 4, wherein said first and second target probes further comprise first and second downstream universal priming sequences, wherein said first and second upstream universal priming sequences and said first and second downstream universal priming sequences flank said first and second adapter sequences, respectively.

10. The method according to claim 9 further comprising contacting said first and second upstream universal priming sequences and said first and second downstream universal priming sequences with first and second universal primers, wherein said amplification is by PCR.

11. The method according to claim 2, wherein said first and second target molecules are proteins.

12. The method according to claim 11, wherein at least said first bioactive agent is an antibody.

13. The method according to claim 11, wherein at least said first bioactive agent is a ligand.

14. The method according to claim 11, wherein at least said first bioactive agent is an aptamer.

15. The method according to claim 11, wherein said first and second upstream universal priming sequences are RNA Polymerase primers and said enzyme is an RNA Polymerase.

16. The method according to claim 15, wherein said first and second upstream universal priming sequences are T7 RNA Polymerase primers.

17. The method according to claim 11, further comprising contacting said first and second upstream universal priming sequences with first and second chimeric RNA/DNA primers, respectively, wherein said amplification is by SPIA.

18. The method according to claim 11, wherein said first and second target probes further comprise first and second downstream universal priming sequences, wherein said first and second upstream universal priming sequences and said first and second downstream universal priming sequences flank said first and second adapter sequences, respectively.

19. The method according to claim 1, wherein said composition further comprises nucleotides.

20. The method according to claim 19, wherein at least said nucleotides are labeled nucleotides.

21. The method according to claim 1, wherein said detecting comprises:

- a. contacting said first and second amplicons with at least one substrate comprising first and second capture probes, wherein said first capture probes are complementary to said first adapters and said second capture probes are complementary to said second adapters; and
- b. detecting said first and second amplicons on said at least one substrate.

22. The method according to claim 21, wherein said substrate comprises an array, said array comprising at least first and second capture probes immobilized on said substrate.

23. The method according to claim 22, wherein said array is an ordered array.

24. The method according to claim 21, wherein said substrate comprises at least a first and a second population of microspheres, wherein said first capture probes are immobilized on said first population of microspheres and said second capture probes are immobilized on said second population of microspheres.

25. The method according to claim 24, wherein said first and second amplicons are detected in a liquid array.

26. The method according to claim 25, wherein said first and second amplicons are detected by FACS.

27. The method according to claim 24, wherein said microspheres are randomly distributed on a second substrate comprising discrete sites.

28. The method according to claim 24, wherein said microspheres are applied to a mass spectrometer and the mass of said adapter sequence is determined to identify the presence of said first and second target molecules.

29. A method for multiplex detection of a plurality of target molecules in a sample said method comprising;

- a. contacting said plurality of target molecules with a composition comprising:
  - i. an amplification enzyme; and
  - ii. a plurality of target probes, each comprising:
    - a) a bioactive agent, wherein each bioactive agent binds to a unique target molecule;
    - b) an adapter sequence that identifies said unique target molecule that binds the bioactive agent; and
    - c) at least one upstream universal priming sequence;

- b. amplifying said adapter sequences using said at least one universal priming sequence, wherein no ligation is performed, to form a plurality of amplicons;
- c. detecting said plurality of amplicons, to indicate the presence or absence of said target molecules in said sample.

- 5      30. The method according to claim 29, wherein said target molecules are selected from the group consisting of proteins and nucleic acids.
31. The method according to claim 30, wherein said target molecules are proteins.
31. The method according to claim 30, wherein said target molecules are nucleic acids.
- 10      32. The method according to claim 29 or 30, wherein said plurality of target molecules comprises at least 500 target molecules.
33. The method according to claim 29 or 30, wherein said plurality of target molecules comprises at least 1000 target molecules.